*Supplementary Information*

# *(S)*-fluoxetine inhibits enterovirus replication by targeting the viral 2C protein in a stereospecific manner

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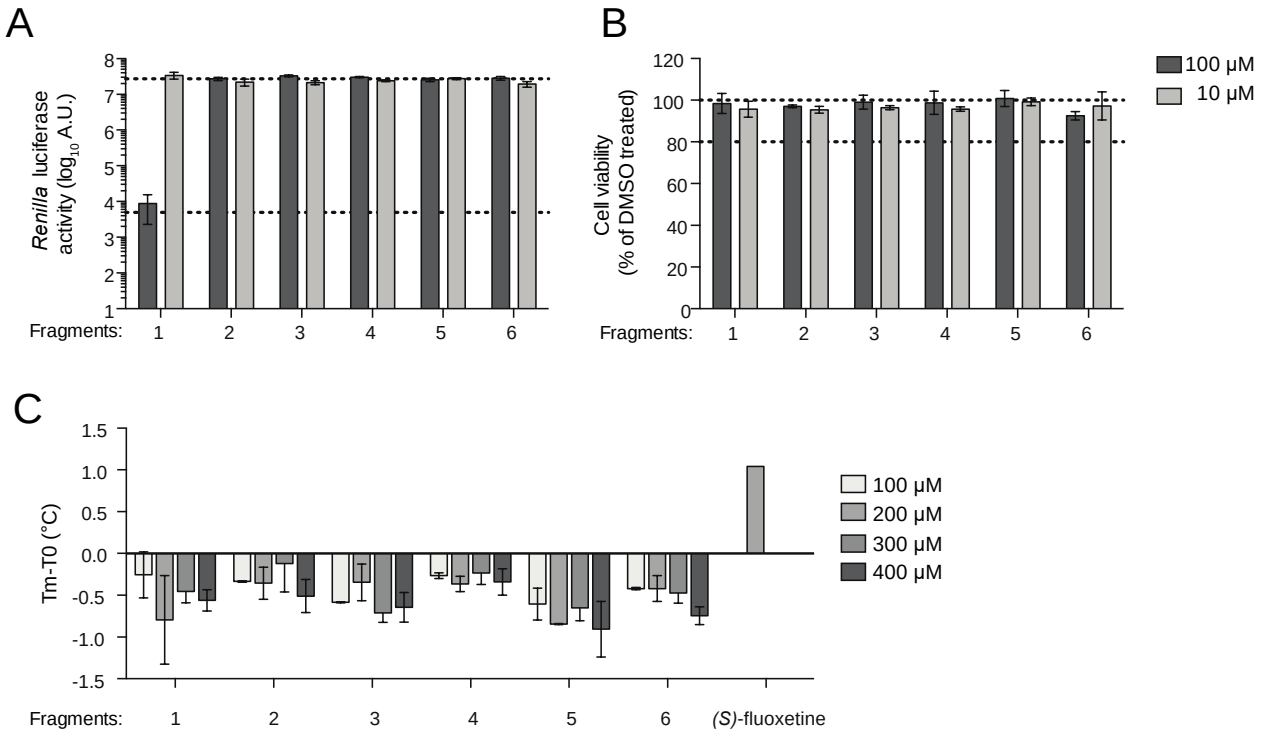
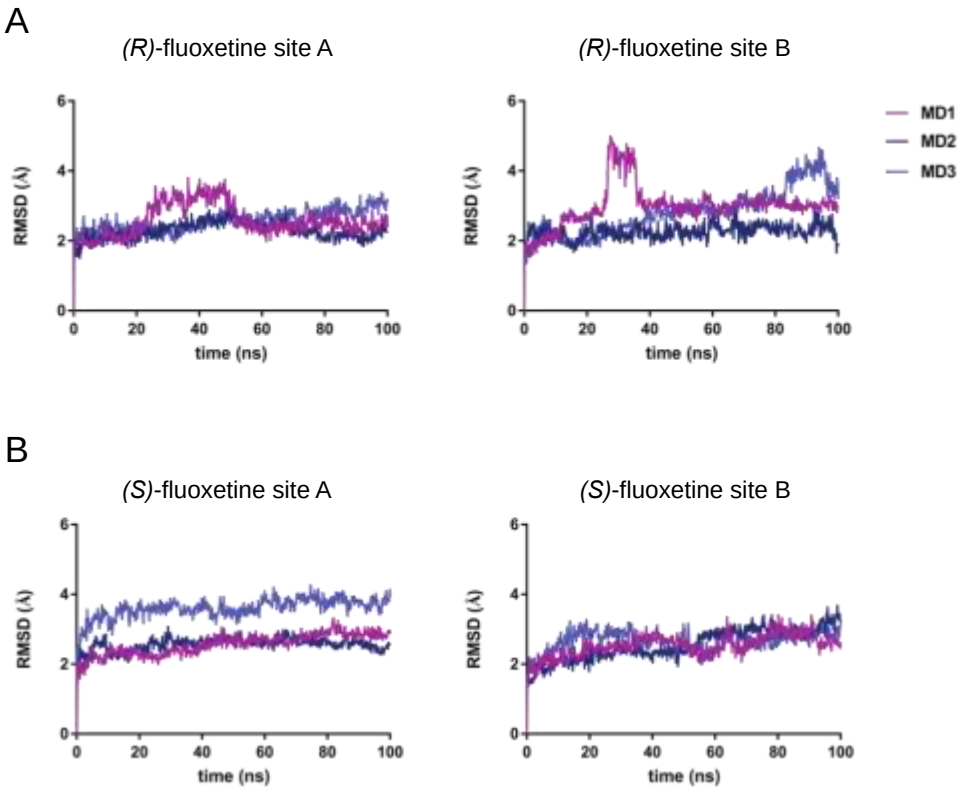
**Table of content:**

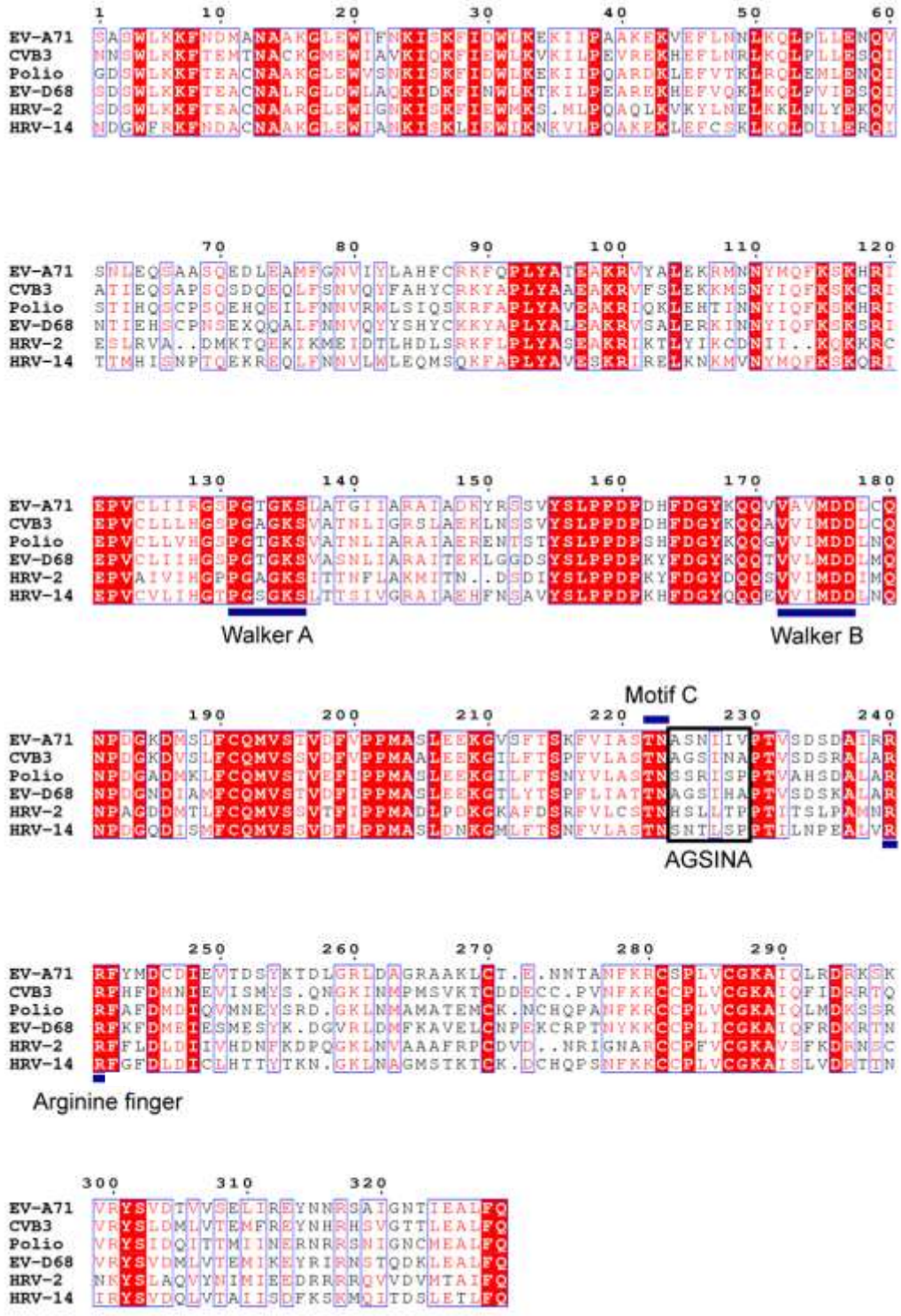



**Supplementary Figure 1. Antiviral activity and binding of fragments to 2C.**

(A) In a single cycle assay, HeLa R19 cells were infected with *Renilla* luciferase (RLuc)-CVB3 reporter virus (MOI 0.1), treated with 100 or 10 µM of fragment, and luciferase activity was determined as a quantitative measure of replication. (B) In parallel, cell viability was determined with an MTS assay. (C) The interaction of the fragments to the 2C protein of CVB3 was determined by thermal shift assays similar as in Figure 1E.




**Supplementary Figure 2. Root mean square deviation (RMSD) of the protein ligand complexes during MD simulations.** Indicator for stability or conformational changes during the simulations. (A) The movement of *(R)*-fluoxetine in site A and site B is shown over 100 ns time during the molecular dynamics. (B) The movement of *(S)*-fluoxetine in site A and site B is shown over 100 ns time during the molecular dynamics.




**Supplementary Figure 3. Multiple sequence alignment of 2C proteins from different enteroviruses.** The multiple sequence alignment of EV-A71, CVB3, Polio-1, EV-D68, HRV-2 and HRV-14 was done with Clustal Omega. Residues in the catalytic center important for ATPase activity are underlined in blue. The 224AGSINA229 region is highlighted in a black box. Conserved residues are highlighted with red background and similar residues are in red letters.